What is good and what is not so good about serological diagnosis of brucellosis

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Good things:

Faster than culturing the organism
More economical
Easily upscaled
Relatively safe
Data can be manipulated
Widely accepted
Tests for every occasion

Not so good things:

Presumptive test – not the ‘gold standard’
Not entirely accurate

- **individual variation in outbred populations**
- **sex, age, gestation etc causes variation**
- **cross reactions**
- **interpretation**
- **vaccination**
- **some tests are better than others**
- **lack of uniformity of protocols**
- **lack of uniformity of reagents**
- **species variation**
- **other stuff**

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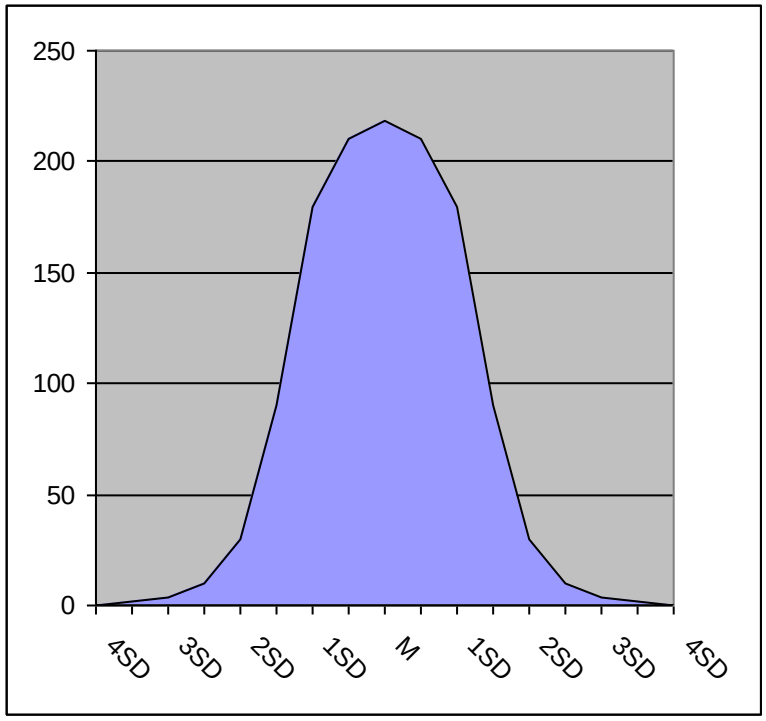

-Standard normal distribution curve
- 95% of the population falls within 2SDs
- 5% are ‘abnormal’, do not fit in
- population on left gives abnormally low values, on right, abnormally high
- makes it impossible to have any biological test 100% accurate
- will always have some animals with high antibody levels and some infected animals with low antibody levels

Age - often older animals respond less vigorously
- young animals respond more strongly

Sex - females are statistically more likely to be persistently infected
- affinity for reproductive organs of females
- latency of infection
- males respond less than females, may underestimate

Gestation - susceptibility increases with pregnancy
- susceptibility increases with time of gestation
- isolation more likely at parturition – 4 fold
- serology may be problematic at parturition

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Cross reactions

The immunodominant epitope on the cell wall of smooth *Brucella sp*. is lipopolysaccharide, specifically O-polysaccharide also known as perosamine

This epitope is found in numerous other microorganisms, including

Yersinia enterocolitica O9

Salmonella urbana

E coli O157

Some *Pasteurella sp.*

Vibrio sp.

Francisella tularensis

And likely others

Cross reactions

Therefore all adult cattle and more than likely most species have a low to substantial level of antibodies to this epitope

Mostly it causes no problems with serological testing as we make allowances for it

But in animals recently exposed to one or more cross reacting microbes, antibody levels may be elevated sufficiently to be problematic

Other antigens from the surface of *Brucella sp*. have not been found to provide consistent serological responses

These include the core regions and lipid A of LPS, omps, various proteins

Vaccination may result in cross reactions that interfere with serology

Interpretation

There are at least 30 serological tests or modifications of serological tests

Many of the test reactions are interpreted by visual inspection resulting in huge variations

Levels of antibody, titers, may be interpreted differently, for example, the complement fixation test is positive at a 1:5 serum dilution in some areas while a 1:40 serum dilution is positive in other areas

Vaccination status of animals may result in different interpretations

Newer tests rely on electronic interpretation of results. This is more accurate but level for positivity varies

No international agreement of positivity of serological reactions

Vaccination

Some vaccines, for example, *Brucella abortus* S19, have the same surface epitopes as pathogenic *Brucella sp.*

A vaccinated animal responds similarly to an infected animal

By vaccinating young animals, antibody levels normally decrease by the time of testing at sexual maturity but not always

By using a reduced vaccine dose, adult animal respond lower interfering less with serology

Vaccines are not interchangeable between species, may cause persistent infection, pathogenic in other species

Vaccines were developed to deal with this problem by deleting the immunodominant epitope, the O-polysaccharide, for example, *B. abortus* RB51

Other subunit or deletion mutant vaccines have not been successful yet

Some tests are better than others

Based on data from cattle

Test	Sensitivity	Specificity	Perf. Index
RBT	81.2	86.2	167.4
BPAT	95.4	97.7	193.1
CFT	89.0	83.5	172.5
IELISA-1	98.6	99.0	197.6
IELISA-2	99.3	99.0	198.3
IELISA-M	100	98.7	198.7
CELISA-1	95.4	100	195.4
CELISA-2	99.5	99.0	198.5
FPA-1	97.5	98.9	196.4
FPA-2	99.9	99.0	198.9

Some tests are better than others

From the table, it is clear that the classical tests, based on antibody performing a secondary reaction, are not quite as accurate

The newer tests, primary binding assays, rely only on the antibody being able to react with its antigen

Most newer tests can be manipulated so that either the sensitivity or the specificity are higher but at a cost

This may be important in control programs

- **in the early stages it is important to detect all infected animals requiring a test of higher sensitivity**
- **in later stages of control, for example, surveillance after eradication, it may be more important to have tests of high specificity to avoid false positive reactions**

Lack of uniformity of protocols

The OIE has attempted to create a uniform test format for some of the commonly used and properly validated serological tests for different species of hosts

These assays are used for international trade but each country usually have their own protocol or SOP, making it difficult to compare data

Some harmonization is taking place by the use of international serum panels and international standards but so far it is very limited

The UM and R (2003 edition) lists the following tests approved by the USDA for use with cattle and bison:

BAPA, RAP, Card, STT, SPT, Rivanol, Manual CFT, Automated CFT, PCFIA, FPA and BRT as well as supplemental tests such as 2ME, Coombs, heat inactivation, IELISA, CELISA, MELISA and CITE

The choice is basically left to the State to decide which of the 18 tests to use

Difference among species

Nearly all investigations of brucellosis serology has been done on cattle because of the economic impact

Some work has been done on small ruminants

Not much has been done on wildlife, however, based on our limited knowledge of domestic animal vaccines used in wildlife, the immune response of most wildlife species is probably sufficiently different from that of domestic animals that serology needs some study

Serological tests used for domestic animals applied to wildlife seems to work, however, as is the case with domestic animal serology, more than one test should be used

Suffice to say, a cow is not a mouse and is not an elk either but you have known this for some time

Other stuff

All those problems and many more not included!!!!

How do we fix things? How can we make an accurate diagnosis using serology?

As it stands, there is no easy fix

Serology using OPS as the main antigen works very well so long as there is no vaccination and no cross reacting microorganisms interfering

In real life, this is not the case

Using the core, lipid A or RLPS may be useful, however, there are some problems with those as well

RLPS is very finicky to make and to use - hydrophobic

- It also seems to give as high as 5% non-specific reactions with normal cattle

- The sensitivity may also leave something to be desired as some infected animals respond below the threshold level

Other stuff

Lipid A is a very poor antigen and not many animals produce antibody to it

The core regions may be useful, however, purified core is difficult to prepare. It is a complex carbohydrate so recombinant technology is not available at this time

It may be useful as it appears to be unique to *Brucella sp*. and it appears that most infected animals produce antibody to it

This scenario many provide a better diagnostic opinion:

Screen with O-polysaccharide antigen (OPS, LPS, Whole cells) - 2 tests

Negative – no further action

Positive OPS reactions, test with core region.

If positive – Brucellosis; if negative - cross reaction

Not 100% effective but…….

Other stuff

	Sensitivity 138 sera	Specificity 1102 sera	Herd test 184 sera	Yersinia 37 sera
RLPS	136 positive	14 positive	183 positive	2 positive
Hydrolyzed RLPS	136 positive	12 positive	183 positive	2 positive
SLPS	137 positive	1 positive	183 positive	35 positive
FPA	137 positive	0 positive	184 positive	16 positive
CFT	121 positive 16 AC	11 positive 38 AC	149 positive 21 AC	31 positive 5 AC

Summary

- **Serology is a presumptive method for demonstrating a high probability of exposure (or lack of exposure) to *Brucella sp.***
- **Things interfere so serology cannot be 100% accurate**
- **Virtually all serological tests available have been developed for diagnosis of brucellosis in domestic animals**
- **We all know a cow is not an elk**
- **The serological tests developed for domestic animals serve as good platforms to start further development**
- **For serious progress to be made in the serological diagnosis in wildlife, some effort must be made to validate tests within the individual species**
- **Difficulties are numerous: gold standard samples, sufficient samples, cross reactions, biochemistry etc.**